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Cyclophosphamide followed by Intravenous Targeted Busulfan for Allogeneic Hematopoietic Cell Transplantation: Pharmacokinetics and Clinical Outcomes

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Targeted busulfan (^{T}BU) and cyclophosphamide (CY) for allogeneic hematopoietic cell transplantation carries a high risk of sinusoidal obstruction syndrome (SOS) in patients undergoing transplantation for myelofibrosis. We tested the hypothesis that reversing the sequence of administration (from ^{T}BU/CY to CY/^{T}BU) would reduce SOS and day +100 nonrelapse mortality. We enrolled 51 patients with myelofibrosis (n = 20), acute myelogenous leukemia (n = 20), or myelodysplastic syndrome (n = 11) in a prospective trial of CY/^{T}BU conditioning for allogeneic hematopoietic cell transplantation. CY 60 mg/kg/day i.v. for 2 days was followed by daily i.v. BU for 4 days, targeted to a concentration at steady state (Css) of 800-900 ng/mL. Compared with ^{T}BU/CY-conditioned patients, CY/^{T}BU-conditioned patients had greater exposure to CY ($P < .0001$) and less exposure to 4-hydroxycyclophosphamide ($P < .0001$). Clinical outcomes were compared between cases and controls (n = 271) conditioned with ^{T}BU/CY for the same indications. In patients with myelofibrosis, CY/^{T}BU conditioning was associated with a significantly reduced incidence of SOS (0% versus 30% after ^{T}BU/CY; $P = .006$), whereas the incidence of SOS was low in both cohorts with acute myelogenous leukemia/myelodysplastic syndrome. Day +100 mortality was significantly lower in the CY/^{T}BU cohort (2% versus 13%; $P = .01$). CY/^{T}BU conditioning had a marked affect on the pharmacokinetics of CY and was associated with significantly lower incidence of SOS and day +100 mortality, suggesting that CY/^{T}BU is superior to ^{T}BU/CY as conditioning for patients with myelofibrosis.

INTRODUCTION

Busulfan (BU) followed by cyclophosphamide (CY) is a commonly used high-dose conditioning regimen in allogeneic hematopoietic cell transplantation (HCT). Regimen-related toxicity, graft rejection, and relapse in patients conditioned with BU/CY have been reduced by individualized dosing of BU to a target steady-state concentration (targeted BU [^{T}BU]) [1,2]. However, neither BU dose targeting nor the introduction of i.v. ^{T}BU has eliminated hepatic sinusoidal obstruction syndrome (SOS) as a cause of morbidity and mortality [3,4]. BU is not inherently toxic to hepatocytes or to sinusoidal endothelial cells, whereas metabolites of CY, generated within hepatocytes and transported into hepatic sinusoids, are highly toxic to sinusoidal endothelial cells [5-7]. It follows that CY metabolites are the prime cause of regimen-related liver toxicity following administration of the ^{T}BU/CY regimen.

There are several possible approaches to minimizing regimen-related toxicity caused by the combination of ^{T}BU and CY. One approach is to eliminate CY altogether by, for example, using a regimen of fludarabine and ^{T}BU [8,9]. A second approach involves eliminating variability of CY exposure with pharmacokinetic targeting of CY doses, which is feasible and effective in reducing toxicity [10]. A third, simpler approach is to reverse the order of administration, giving CY first, followed by i.v. ^{T}BU (CY/^{T}BU). The pharmacologic rationale for a CY/^{T}BU regimen rests on the following observations: (1) BU depletes hepatic glutathione and at high concentrations induces oxidative stress in murine hepatocytes in vitro [6]; (2) glutathione is important in both the detoxification of the CY metabolite 4-hydroxycyclophosphamide (HCY) through conversion to glutathionyl-CY and in the elimination of the toxic CY metabolite acrolein [5,11]; (3) restoration of hepatic and sinusoidal endothelial cell glutathione levels prevents injury to hepatic sinusoids in several different animal models of toxic liver injury [12]; and (4) studies in patients receiving high-dose conditioning regimens have suggested a reduced risk of hepatotoxicity when BU is given after, rather than before, other conditioning agents [13-15]. Thus, giving BU first appears to potentiate CY toxicity, providing the basis for administering these drugs in reverse order (CY/^{T}BU) to reduce toxicity.

Here we report the results of a prospective clinical trial designed to test the hypothesis that reversed-sequence (CY/^{T}BU) conditioning reduces the frequency and severity of hepatotoxicity compared with the standard sequence of BU followed by CY (^{T}BU/CY). In addition, we collected pharmacokinetic data to test whether altering the sequence of conditioning agents led to measurable changes in CY metabolism and exposure to CY metabolites. We enrolled 2 cohorts of patients, 1 cohort at high risk for toxic sinusoidal liver injury (patients with myelofibrosis) [16] and the other at standard risk (patients with myelodysplastic syndrome

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[MDS] or acute myelogenous leukemia [AML]). We compared liver toxicity and outcomes with those in concurrent and historical control patients who received ^{T}BU/CY and allogeneic HCT for the same disease indications. The primary outcome was the incidence of moderate/severe SOS after allogeneic HCT.

MATERIALS AND METHODS

Patient Selection

Study patients (cases) were enrolled from March 1, 2007, through June 30, 2010, on Fred Hutchinson Cancer Research Center (FHCRC) Protocol 2130. This protocol was approved by the FHCRC Institutional Review Board and registered at www.clinicaltrials.gov as NCT00445744. All patients provided written informed consent using forms approved by the Institutional Review Board. Under the aegis of Institutional Review Board–approved Protocol 881, a cohort of historical patients (controls) was obtained by retrieving clinical data on consecutive patients with myelofibrosis, AML, or MDS undergoing allogeneic HCT after ^{T}BU/CY conditioning between January 1, 2003, and December 31, 2009.

Eligibility Criteria

Eligibility criteria included (1) presence of primary myelofibrosis, myelofibrosis secondary to polycythemia vera or essential thrombocythemia, AML, or MDS; (2) age <61 years if receiving a transplant from an unrelated donor or age <66 years if receiving a transplant from a related donor; (3) receipt of unmanipulated granulocyte colony stimulating factor–mobilized peripheral blood mononuclear cells (G-PBMCs) or granulocyte colony stimulating factor–stimulated bone marrow allograft products; (4) Karnofsky performance status of >70% at the time of HCT; and (5) ability to provide informed consent. Patients were required to have an HLA-identical related donor or an HLA-matched or 1 HLA allele–mismatched unrelated donor identified before enrollment.

Exclusion criteria included (1) HIV infection or active viral hepatitis; (2) use of medications known to strongly inhibit the cytochrome P450 pathway that could not be safely discontinued during conditioning, in the judgment of the attending physician; (3) known hypersensitivity to BU or CY; (4) hepatic dysfunction, as evidenced by total serum bilirubin or aspartate aminotransferase more than 2 times the upper limit of normal, or evidence of synthetic dysfunction or cirrhosis; (5) renal insufficiency, as evidenced by creatinine clearance <50% of expected, serum creatinine more than 2 times the upper limit of normal, or dialysis dependence; (6) impaired pulmonary function, as evidenced by arterial partial pressure of oxygen (PaO_2) <70 mm Hg and diffusing capacity of the lung for carbon monoxide (DLCO) <70% of predicted or by PaO_2 <80 mm Hg and DLCO <60%, or requirement for continuous supplementary oxygen; and (7) impaired cardiac function, as evidenced by an ejection fraction <35% or the presence of symptomatic coronary artery disease.

Conditioning Regimen

The conditioning regimens for protocol cases and control patients are summarized in Table 1. All patients were conditioned with CY 60 mg/kg/day i.v. for 2 consecutive days (total dose, 120 mg/kg) and targeted BU, given for 4 consecutive days. On the days of CY infusion, patients received 2-mercaptoethane sulfonate at milligram doses equal to those of CY as prophylaxis against uroepithelial damage.

Cases (n = 51) received CY followed by ^{T}BU (CY/^{T}BU). CY was administered i.v. at 60 mg/kg/day on days −7 and −6 before HCT. ^{T}BU was administered i.v. as Busulfex (Otsuka Pharmaceutical, Tokyo, Japan) once daily on days −5 through −2, for a total of 4 daily doses. Prophylactic phenytoin was initiated on day −6 after completion of the second CY dose and discontinued on day −1. One patient received prophylactic levetiracetam.

Patients in the control cohort (n = 271) received ^{T}BU followed by CY (^{T}BU/CY). In this cohort, BU was administered orally on days −7 through −4 at an initial dose of 1 mg/kg every 6 hours in 252 patients (93%), i.v. at a starting dose of 0.8 mg/kg every 6 hours in 15 patients (6%), and i.v. at a starting dose of 3.2 mg/kg daily in 4 patients (1%). After the initial weight-based dose of BU, subsequent doses were adjusted to achieve the target plasma steady-state concentrations (Css) shown in Table 1. CY was administered at 60 mg/kg/day i.v. on days −3 and −2. Prophylactic phenytoin was given from day −8 through day −3.

CY Dosing and Pharmacokinetics

CY was infused through a central venous catheter. The CY dose was based on adjusted ideal body weight (0.25 × [actual weight − ideal weight] + ideal weight) if actual body weight was greater than ideal body weight [17]. The infusion duration followed FHCRC Standard Practice Guidelines; total CY doses of <5000 mg were infused over 1 hour, and doses ≥5000 mg were infused over 2 hours. The CY dose was not adjusted based on pharmacokinetic data.

Table 1
Conditioning Regimens for Cases (CY/^{T}BU) and Controls (^{T}BU/CY)

	CY/^{T}BU Cases (n = 51)	^{T}BU/CY Controls (n = 271)
Conditioning agents, by transplantation day		
−7	CY i.v. 60 mg/kg*	BU†
−6	CY i.v. 60 mg/kg	^{T}BU
−5	BU[c]	^{T}BU
−4	^{T}BU	^{T}BU
−3	^{T}BU	CY i.v. 60 mg/kg*
−2	^{T}BU	CY i.v. 60 mg/kg
−1	Rest	Rest
0	Allograft infusion	Allograft infusion
BU administration route and dosing frequency, n (%)		
Oral every 6 h	0	252 (93)
i.v. every 6 h	0	15 (6)
i.v. once daily	51 (100)	4 (1)
Cumulative BU dose, mg, median (range)		
Oral	Not applicable	1048 (572-1916)
i.v.	1098 (580-1510)	976 (608-1668)
Target BU Css, ng/mL, n (%)		
≤900‡	0	2 (0.7)
600-900	0	3 (1.1)
800-900	51 (100)	262 (96.7)
>900‡	0	4 (1.5)
BU pharmacokinetics		
First dose Css >900 ng/mL, n (%)	23 (45)	128 (47)
Average daily Css >900 ng/mL, n (%)§	1 (2)	18 (7)
Average daily Css, ng/mL, median (range)§	856 (811-1191)	861 (627-968)

BU indicates busulfan; ^{T}BU, targeted busulfan; CY, cyclophosphamide; Css, steady-state concentration.

* 2-Mercaptoethane sulfonate was given concurrently with i.v. CY to prevent hemorrhagic cystitis.

† Phenytoin was started 1 day before ^{T}BU and continued throughout ^{T}BU administration (ie, day −6 through day −1 for CY/^{T}BU and day −8 through day −3 for ^{T}BU/CY).

‡ Specific target Css detailed in the text.

§ Cumulative over all 4 days of ^{T}BU administration. For Css, each patient's ^{T}BU Css over all 4 days was calculated, and then divided by 4 to provide the average daily Css.

In cases (CY/^{T}BU) only, blood samples were drawn after each dose of CY from the central venous line at 2, 4, 8, 16, 20, and 24 hours after the start of CY infusion and at the cessation of CY infusion. If the CY infusion lasted 1.5 hours or longer, then blood samples were instead drawn at 3, 5, 8, 16, 20, and 24 hours after the start of CY infusion and at the cessation of CY infusion. At each of these time points, blood was aliquoted into 2 tubes, 1 tube containing EDTA for analysis of CY and carboxyethylphosphoramide mustard (CEPM) and the other containing phenylhydrazine HCl to stabilize HCY, as described previously [18]. Samples were refrigerated at the bedside at a target temperature of 4° C until being transported (within 12 hours) to the pharmacokinetics laboratory. Plasma concentrations of CY, HCY, and CEPM were quantified by liquid chromatography mass spectroscopy methods [10]. A patient's exposure to CY and its metabolites was calculated by determining the area under the curve (AUC) for CY (AUC_{CY}), for HCY (AUC_{HCY}), and for CEPM (AUC_{CEPM}) for interval from 0 to 48 hours using noncompartmental analysis. These AUCs were compared with those previously reported in patients receiving ^{T}BU/CY [18]. CY pharmacokinetics were not evaluated in the historical control patients.

BU Dosing and Pharmacokinetics

In the 51 cases (CY/^{T}BU), daily i.v. BU doses were standardized in terms of time of administration, duration of infusion, and administration of saline flushes in the i.v. line, to ensure consistent BU pharmacokinetics. In these patients, the first BU dose (day −5) was 4 mg/kg, with body weight calculated as described above [17]. All subsequent BU doses were adjusted to achieve a Css of 800-900 ng/mL.

In the 271 control patients (^{T}BU/CY), the BU administration route and target Css were chosen by the attending physician. The majority of patients received oral BU every 6 hours (n = 252); a minority received i.v. BU every

6 hours (n = 15) or as a combined single daily dose (n = 4). The target Css for most patients (n = 262) was 800-900 ng/mL; 5 patients had target Css ≤900 ng/mL, and 4 patients had a target Css >900 ng/mL.

In both cases and controls, blood samples for BU pharmacokinetics (3 mL/sample) were collected in sodium heparin–containing tubes at the time points described previously [8]. Samples were stored on wet ice or refrigerated until being transported to the laboratory, where plasma BU concentrations were analyzed by gas chromatography with mass selective detection as described previously [19]. The dynamic range was 62-4500 ng/mL, and the intraday and interday coefficients of variation were <5% and <8%, respectively.

Individual patient concentration-time data were fit using WinNonlin version 5.2 (Pharsight, Sunnyvale, CA). The AUC from time 0 to infinity ($AUC_{0\ to\ \infty}$) was calculated after each dose. Clearance and Css were calculated based on the following equations:

$$\text{Clearance} = \text{dose} \div \text{AUC}$$

$$\text{Css} = AUC_{0\ to} \times \text{BU molecular weight (246.3 g/mol)} \div \text{dosing interval.}$$

After calculation of each patient's clearance, subsequent dose levels were calculated linearly to achieve the target Css, as described previously [17].

Supportive Care and Prophylaxis

Graft-versus-host disease (GVHD) prophylaxis consisted of tacrolimus and methotrexate. Tacrolimus was given as a continuous i.v. infusion beginning on day −1 at an initial dose of 0.03 mg/kg/day, with doses adjusted to achieve a trough tacrolimus Css of 5-15 ng/mL. Tacrolimus admininstration was changed from i.v. infusion to divided oral dosing as soon as could be tolerated. In the absence of GVHD, tacrolimus was tapered in 20% decrements starting on day +56 after HCT and discontinued completely by day +200. In patients with GVHD, tacrolimus was maintained at therapeutic trough concentrations, with subsequent tapering and management dictated by the attending transplantation physician on the basis of clinical GVHD activity. Methotrexate was given at a dose of 10 mg/m^2 i.v. on day +1 (at least 24 hours after donor cell infusion) and on days +3, +6, and +11.

All patients received antifungal, antiviral, and antibacterial prophylaxis in accordance with standard practice at FHCRC. Hematopoietic growth factors were given only in the event of prolonged neutropenia after day +21. Ursodiol was administered orally to both cases and historical controls at 12 mg/kg/day, starting 2 weeks before the initiation of conditioning, again in accordance with FHCRC standard practice.

Evaluation of Outcomes

All case and control patients were evaluated by 2 investigators (G.B.M. and A.K.) for evidence of SOS after HCT. The diagnosis of SOS was based on the presence of at least 2 of the following features by day +20 after HCT: hyperbilirubinemia (ie, serum bilirubin >2.0 mg/dL), hepatomegaly or right upper quadrant pain of liver origin, and weight gain (>2% of dry body weight) due to fluid accumulation [20]. Patients with other possible causes of liver dysfunction (eg, GVHD, sepsis syndrome, drug-induced liver injury) were classified as having liver disease of uncertain etiology. The severity of SOS was graded as mild (resolving without specific treatment), moderate (requiring diuretics, sodium restriction, or analgesics, but with eventual resolution of abnormalities), or severe (death or nonresolution by day +100).

Overall survival was estimated by the Kaplan-Meier method. The cumulative incidences of nonrelapse mortality (NRM) and relapse were estimated by standard methods, treating these outcomes as mutually competing events. Statistical comparisons of survival, NRM, and relapse between groups used Cox regression, restricting the analysis to events within the first 100 days or first 2 years after HCT, as indicated. The associations of AUC_{CY} and its metabolites with these outcomes were evaluated as a test for trend over quartiles using Cox regression. Statistical comparisons of the frequency of SOS were done using the chi-squared test. Pharmacokinetic parameters were compared between regimens using the Wilcoxon rank-sum test. Comparisons of relapse rates were adjusted using the disease risk criteria described by Kahl et al. [21]. Outcomes in patients with AML/MDS were compared with those in patients with myelofibrosis as part of a prespecified subset analysis.

RESULTS

Patient Characteristics

Patient and disease characteristics are summarized in Table 2. The median age of cases was 55 years (range, 30 to 65 years). Twenty case patients (39%) had myelofibrosis, 11 (22%) had MDS, and 20 (39%) had AML. Two patients had undergone previous allogeneic HCT; 1 patient, with myelofibrosis, had rejected an allograft after ^{T}BU/CY conditioning 10 years earlier, and the other, with AML, had relapsed after HCT with reduced-intensity conditioning performed 3 months before study enrollment. The median age in the control cohort of 271 patients was 50 years (range, 19 to 67 years). In the control cohort, 33 patients (12%) had myelofibrosis, 143 (53%) had AML, and 95 (35%) had MDS.

Table 2
Patient Characteristics

Characteristic	CY/^{T}BU Cases (n = 51)	^{T}BU/CY Controls (n = 271)
Age, years, median (range)	55 (30-65)	50 (19-67)
Diagnosis, n (%)		
Acute myelogenous leukemia	20 (39)	143 (53)
Myelodysplasia	11 (22)	95 (35)
Myelofibrosis	20 (39)	33 (12)
Donor, n (%)		
Related	28 (55)	96 (35)
Unrelated	23 (45)	175 (65)
HLA-matched	21	98
One HLA allele-mismatched	2	77
Allograft source, n (%)		
G-PBMCs	51 (100)	223 (83)
Bone marrow	0 (0)	48 (17)
$CD34^{+}$ dose, cells/kg recipient weight, median (range)	13.4 (6.8-28.5)	9.1 (0.5-45.0)
Kahl disease risk, n (%)*		
Low/moderate	30 (59)	196 (72)
High	21 (41)	75 (28)

CY indicates cyclophosphamide; ^{T}BU, targeted busulfan; G-PBMCs, granulocyte colony-stimulating factor-mobilized peripheral blood mononuclear cells.

* Kahl disease risk reflects the risk of relapse after allogeneic hematopoietic cell transplantation [21].

Cyclophosphamide Pharmacokinetics

Peak plasma concentrations and AUC_{CY}, AUC_{HCY}, and AUC_{CEPM} are summarized in Table 3 and Figure 1. The patients receiving CY/^{T}BU showed considerable variability in exposure to CY metabolites, including a 3.7-fold variation in AUC_{CY}, a 3.6-fold variation in AUC_{HCY}, and a 4.8-fold variation in AUC_{CEPM}. Pharmacokinetic parameters for patients receiving CY/^{T}BU were compared with those obtained previously for 75 patients who received ^{T}BU/CY conditioning [18]. These analyses were adjusted for patient age, given the

Table 3
Comparison of Pharmacokinetics of CY, HCY, and CEPM by Conditioning Regimen

	CY/^{T}BU	^{T}BU/CY [18]	*P* Value
CY			
Peak [CY], day 1	375 ± 60	312 ± 171	<.0001
Peak [CY], day 2	329 ± 66	283 ± 124	<.0001
AUC_{CY}	4899 ± 1255	2563 ± 1190	<.0001
HCY			
Peak [HCY], day 1	9 ± 5	35 ± 18	<.0001
Peak [HCY], day 2	20 ± 9	36 ± 13	<.0001
AUC_{HCY}	168 ± 48	290 ± 98	<.0001
CEPM			
Peak [CEPM], day 1	12 ± 6	27 ± 12	<.0001
Peak [CEPM], day 2	26 ± 11	32 ± 29	.25
AUC_{CEPM}	475 ± 180	522 ± 194	.14

CY indicates cyclophosphamide; ^{T}BU, targeted busulfan; AUC, area under the curve; HCY, 4-hydroxycyclophosphamide; CEPM, carboxyethylphosphoramide mustard. Peak concentrations (μM) are the highest concentrations recorded on that day. AUC (μM·h) is from time 0 to 48 hours. Comparisons are adjusted for age.

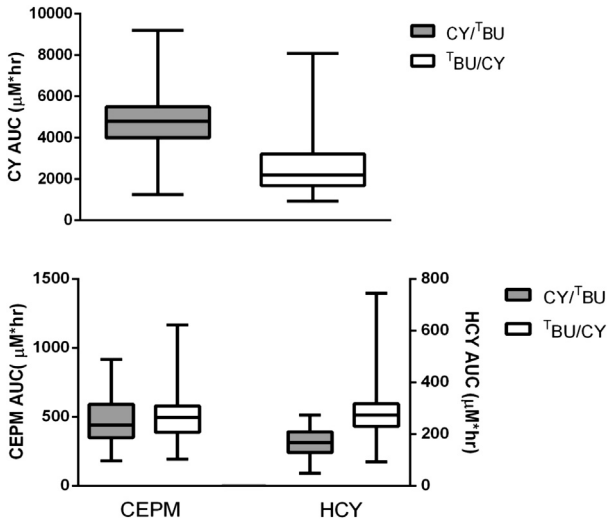

Figure 1. Comparison of CY, HCY, and CEPM exposure by conditioning regimen. $AUC_{0\text{-}48hr}$ in patients receiving CY/^{T}BU (gray) and ^{T}BU/CY (white). Boxes designate 25th, 50th, and 75th percentiles; whiskers designate 5th and 95th percentiles.

age-dependent pharmacokinetics of CY [22]. The median patient age was 55 years (range, 30 to 65 years) in the CY/^{T}BU cohort and 44 years (range, 20 to 66 years) in the historical ^{T}BU/CY cohort [18].

The sequence of CY/^{T}BU administration had a marked affect on CY pharmacokinetics (Table 3 and Figure 1). When CY was given first (CY/^{T}BU), there was a significant increase in AUC_{CY} (4899 versus 2563 mU·h; $P < .0001$) and a significant decrease in AUC_{HCY} (168 versus 290 mU·h; $P < .0001$) compared with values seen with standard ^{T}BU/CY. There was also a trend toward lower AUC_{CEPM} with CY/^{T}BU (475 versus 522 mU·h, $P = .14$). In the CY/^{T}BU cohort, there were no apparent differences in BU Css or in the AUC of CY and its metabolites between patients with myelofibrosis and those with AML/MDS (data not shown). In the CY/^{T}BU cohort, associations of AUC_{CY}, AUC_{HCY}, and AUC_{CEPM} with SOS could not be evaluated statistically, because only 2 cases of SOS occurred. Relapse and NRM were not associated with AUC_{CY}, AUC_{HCY}, and AUC_{CEPM}; however, higher AUC_{HCY} and AUC_{CEPM} were associated with inferior overall survival ($P = .03$ and .02, respectively) (Table 4).

Clinical Outcomes in Cases

All patients in the CY/^{T}BU cohort initially engrafted (defined as a rise in absolute neutrophil count to >500 cells/μL for at least 3 consecutive days) at a median of 17 days (range, 11 to 30 days) after HCT. One patient with AML/MDS who received an HLA allele–mismatched allograft from an unrelated donor suffered late graft failure at 3 months after HCT.

Approximately one half of cases (26 of 51; 51%) did not require parenteral nutrition within the first 20 days after allogeneic HCT. In patients with myelofibrosis, the median peak serum total bilirubin level through day +20 was 2.3 mg/dL (range, 0.7 to 30.0 mg/dL). In patients with AML/MDS, the median peak serum total bilirubin level through day +20 was 1.1 mg/dL (range, 0.5 to 12.4 mg/dL). The incidence of SOS was 0 of 20 (0%) in patients with myelofibrosis and 2 of 31 (6.5%) in patients with AML/MDS (Table 5). No patient in the CY/^{T}BU cohort developed severe SOS.

Acute GVHD grade II-IV and grade III-IV occurred in 67% and 8% of cases, respectively, at a median of 28 days (range, 8 to 102 days) after HCT. Chronic GVHD developed in 41% of cases, at a median of 189 days (range, 92 to 530 days) after HCT.

Table 4
Relationship between Exposure to CY and Its Metabolites and Clinical Outcomes among Cases Conditioned with CY/^{T}BU*

Clinical Outcome	n†	Pharmacokinetic Parameters, HR (*P* Value)		
		AUC_{CY}	AUC_{HCY}	AUC_{CEPM}
Nonrelapse mortality	10	1.15 (.64)	1.67 (.11)	1.40 (.25)
Relapse	9	1.05 (.84)	1.2 (.53)	1.53 (.10)
Overall mortality	20	1.28 (.26)	1.74 (.03)	1.67 (.02)

CY indicates cyclophosphamide; ^{T}BU, targeted busulfan; HR, hazard ratio; AUC, area under the curve; HCY, 4-hydroxycyclophosphamide; CEPM, carboxyethylphosphoramide mustard.

* AUC is modeled as a continuous linear variable, with HRs for AUC_{CEPM} and AUC_{HCY} representing an increase in HR associated with an increase in AUC of 100 μM·h. HRs for AUC_{CY} represent an increase in hazard associated with increase in AUC of 1000 μM·h. HRs are adjusted for age at time of HCT, type of donor, and relapse risk.

† Number of events in cohort in 51 cases.

The median follow-up of surviving cases was 19 months, and 32 patients (63%) were alive at last follow-up. Day +100 mortality was 0% in patients with myelofibrosis and 3% in those with AML/MDS. At 2 years after HCT, cumulative incidence estimates for overall survival were 68% in patients with myelofibrosis and 56% in those with AML/MDS, and cumulative incidence estimates for NRM were 27% in patients with myelofibrosis and 17% in those with AML/MDS. The cumulative incidence of relapse was 11% in patients with myelofibrosis and 44% in patients with AML/MDS.

The major causes of death were relapsed malignancy in patients with AML/MDS and GVHD (with or without concomitant infection) in patients with myelofibrosis. One patient with myelofibrosis died of metastatic prostate cancer, which was diagnosed approximately 6 months after HCT. One patient with AML/MDS committed suicide at day +102 after HCT.

Comparison of Outcomes after CY/^{T}BU versus ^{T}BU/CY

Among patients with myelofibrosis, CY/^{T}BU conditioning was associated with a significantly reduced incidence of SOS compared with ^{T}BU/CY conditioning (0% versus 30%; $P = .006$). In patients with AML/MDS, the SOS rate was 6.5% with CY/^{T}BU and 9.2% with ^{T}BU/CY ($P = .61$). There were no cases

Table 5
Incidence of Sinusoidal Obstruction Syndrome and Liver Disease of Unknown Etiology in Patients Conditioned with ^{T}BU/CY Versus CY/^{T}BU

	CY/^{T}BU (n = 51)	^{T}BU/CY (n = 271)
Myelofibrosis, n (%)	20	33
No liver disease	17 (85)	19 (58)
LDUE	3 (15)	4 (12)
SOS	0 (0)	10 (30)
Mild	0	2
Moderate	0	6
Severe	0	2
AML/MDS, n (%)	31	238
No liver disease	26 (84)	203 (85)
LDUE	3 (10)	13 (5)
SOS	2 (6)	22 (9)
Mild	0	3
Moderate	2	10
Severe	0	9

CY indicates cyclophosphamide; ^{T}BU, targeted busulfan; LDUE, liver disease of unknown etiology; SOS, sinusoidal obstruction syndrome; AML, acute myelogenous leukemia; MDS, myelodysplastic syndrome.

of severe SOS in the CY/^{T}BU cohort, compared with 11 cases in the ^{T}BU/CY cohort.

In patients with myelofibrosis, median peak serum total bilirubin levels through day +20 did not differ significantly by conditioning agent sequence (2.3 mg/dL in the CY/^{T}BU group versus 2.2 mg/dL in the ^{T}BU/CY group; $P = .95$). In patients with AML/MDS, the CY/^{T}BU group showed a trend toward a lower median peak serum total bilirubin level through day +20 (1.1 mg/dL versus 1.4 mg/dL; $P = .07$).

Patients conditioned with CY/^{T}BU had significantly lower day +100 mortality compared with those conditioned with ^{T}BU/CY (2% versus 12%; $P = .01$). For patients with myelofibrosis, the 2-year cumulative incidence of relapse was 11% in the CY/^{T}BU group and 6% in the ^{T}BU/CY group ($P = .62$). There were no significant between-group differences in the 2-year cumulative incidence of NRM (27% versus 25%; $P = .91$) or overall survival (68% versus 72%; $P = .78$) (Figure 2).

For patients with AML/MDS, the 2-year cumulative incidence of relapse was 44% with CY/^{T}BU versus 20% with ^{T}BU/CY ($P = .008$); the 2-year cumulative incidence of NRM was 17% versus 22% ($P = .84$), and the 2-year cumulative incidence of overall survival was 56% versus 64% ($P = .57$) (Figure 3). The higher incidence of relapse in patients with AML/MDS conditioned with CY/^{T}BU remained statistically significant, albeit attenuated, after adjustment for the greater disease risk in this cohort (unadjusted hazard ratio [HR], 2.57, $P = .008$; adjusted HR, 2.15, $P = .02$).

DISCUSSION

The major findings of this study are as follows: (1) Daily i.v. BU can be safely administered after high-dose CY; (2) the sequence of BU and CY administration has a significant effect on CY metabolism; and (3) compared with the standard sequence of ^{T}BU/CY, CY/^{T}BU conditioning is associated with a significantly reduced risk of day +100 mortality, a significantly lower incidence of SOS, and the absence of severe SOS. In patients with myelofibrosis, the decrease in SOS incidence from 30% to 0% with a simple reversal of conditioning agent sequence is both statistically and clinically significant. BU is not inherently hepatotoxic as a single agent in vitro or in human overdoses [6,23]. Our data reinforce the idea that regimen-related liver damage results largely from toxic metabolites of CY, although we recognize reports of hepatotoxicity attributed to BU in combination with fludarabine as well [24,25].

Recent reports support the safety of daily i.v. BU with CY [26,27]. However, Williams et al. [28] concluded that daily i.v. BU at 3.2 mg/kg/day for 4 days followed by CY 60 mg/kg/day for 2 days resulted in excessive toxicity; autopsy-confirmed SOS occurred in 2 of their 3 patients who received this regimen, with a BU Css of >1025 ng/mL. Our cases received the same dose of CY, but in the reverse sequence, followed by daily i.v. BU at a higher initial dose of 4 mg/kg, with subsequent BU doses targeted to a Css of 800-900 ng/mL. This target BU Css range is well below the BU Css ranges of 925 to 1025 ng/mL previously associated with elevated SOS rates in adults conditioned with BU/CY [2,29,30]. Our data demonstrate acceptable toxicity when CY is administered before daily i.v. ^{T}BU at an initial BU dose of 4 mg/kg. Notably, the clearance of daily i.v. BU did not change during days −5 to −3 in patients receiving CY/^{T}BU [17]. Nevertheless, even in patients receiving CY/^{T}BU, CY metabolism showed substantial interpatient variability when the CY dose, phenytoin dose, and BU Css were held constant among patients (Table 3 and Figure 1).

Patients receiving CY/^{T}BU had greater exposure to CY, lower exposure to HCY, and similar exposure to CEPM compared with ^{T}BU/CY-conditioned patients. These data agree with our previous report comparing CY/total body irradiation (ie, CY first) and ^{T}BU/CY (ie, CY after BU/phenytoin) [18]. The use of phenytoin as a prophylactic antiepileptic may contribute to this difference. We sought to characterize the clinical significance of the variability in CY pharmacokinetics (Table 4). Theoretically, reduced HCY exposure may translate into less immunosuppressive effect, although AUC_{HCY} was not associated with clinical outcomes in patients conditioned with

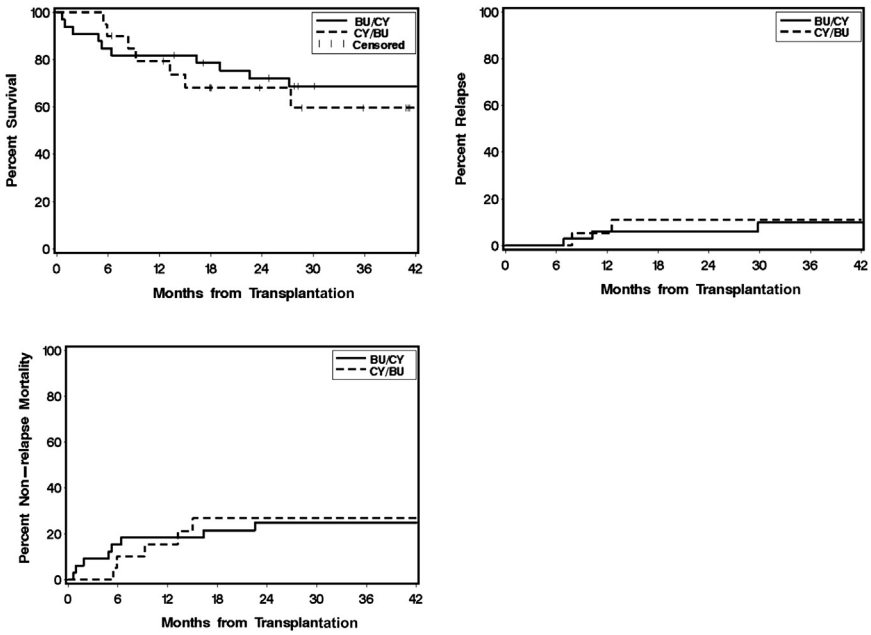

Figure 2. Overall survival, NRM, and relapse in patients with myelofibrosis conditioned with CY/^{T}BU (n = 20) versus those conditioned with ^{T}BU/CY (n = 33).

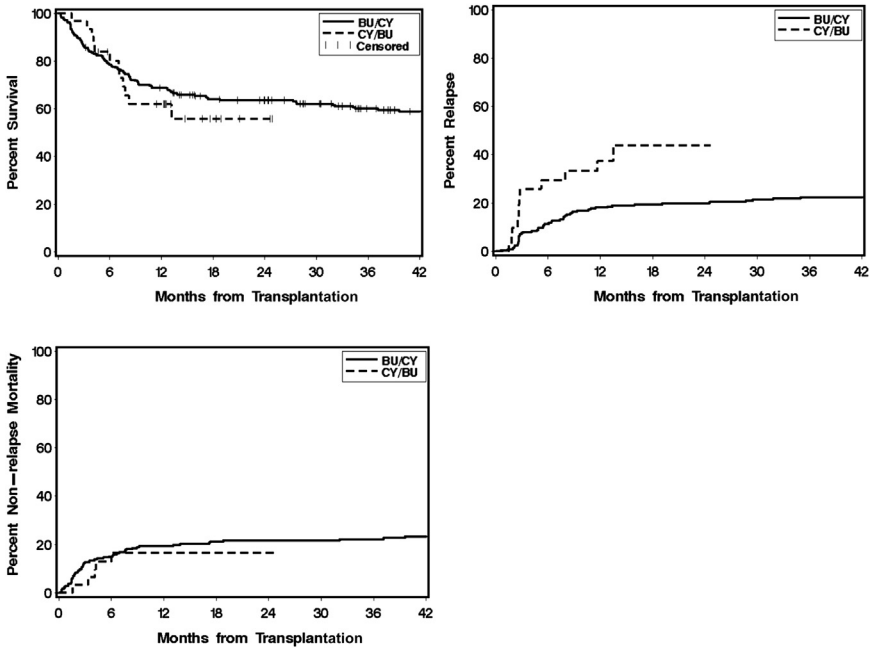

Figure 3. Overall survival, NRM, and relapse in patients with AML/MDS conditioned with CY/^{T}BU (n = 31) versus those conditioned with ^{T}BU/CY (n = 238).

CY/total body irradiation or ^{T}BU/CY [7,18]. In terms of toxicity, we previously described AUC_{CEPM} as a reporter for liver toxicity, with a strong correlation with sinusoidal hepatotoxicity and mortality [7,22]. We could not perform a pharmacodynamic analysis of SOS with CY metabolites, because only 2 of the CY/^{T}BU cases developed SOS. We found statistically significant relationships between overall survival and AUC_{HCY} and AUC_{CEPM} (Table 4).

Altering the sequence of conditioning agents is an appealingly simple and inexpensive strategy that uses readily available and familiar medications. Following preclinical studies [5,31,32], this approach has been translated into clinical trials in humans. Kerbauy et al. [14] evaluated the use of CY/BU conditioning in a cohort of 11 patients and reported lower peak serum aminotransferase levels compared with BU/CY-conditioned historical controls [14]. Of note, peak serum total bilirubin levels were not significantly different in the 2 groups. In a larger retrospective study, Cantoni et al. [15] reported lower rates of SOS and transplantation-related mortality in a cohort of 59 patients conditioned with CY/BU compared with a small historical cohort of 16 patients conditioned with BU/CY.

Our results extend the previous retrospective reports in the form of a prospective clinical trial. In addition to prospective enrollment, novel aspects of the present study include a focus on patients at high risk for hepatotoxicity (ie, those with myelofibrosis), the availability of a large cohort of concurrent control patients conditioned with ^{T}BU/CY, the determination of CY pharmacokinetics, and pharmacokinetic BU targeting to rule out variability in BU exposure as a confounding factor. The target plasma BU Css was 800-900 ng/mL for 100% of the cases (i.v. BU) and 96.7% of the controls (oral and i.v. BU). Intravenous BU has been associated with reduced hepatotoxicity compared with oral BU when dosed by body weight [33]. However, when BU dosing is personalized to a target steady-state concentration, as in the present study, outcomes appear to be similar regardless of administration route [26]. Thus, given the consistent pharmacokinetic targeting of BU in our case and control patients, the route of administration is unlikely to account for the observed differences in outcomes.

We found a greater risk of relapse in patients with AML/MDS conditioned with CY/^{T}BU compared with those conditioned with ^{T}BU/CY. Some of this risk may be related to confounding variables; patients at high baseline risk of relapse were over-represented in the case cohort, and the relapse rate in the control cohort (20%) was somewhat lower than that generally reported in the literature [34]. Nonetheless, we cannot rule out the possibility that reversing the sequence of conditioning agents may increase the risk of relapse in patients with AML/MDS. Thus, our data do not support the use of this regimen to treat AML/MDS outside the confines of a well-designed clinical trial.

The major limitation of the present study is our use of a concurrent/historical control cohort rather than prospective randomization between the CY/^{T}BU and ^{T}BU/CY arms. Our control cohort contained a higher proportion of patients receiving bone marrow (as opposed to G-PBMC) allografts. However, given that the most recent available data suggest equivalent outcomes with bone marrow and G-PBMC allografts [35], this discrepancy is unlikely to be a significant source of bias in terms of the clinical outcomes of interest. Similarly, our control cohort contained a larger number of patients with HLA-mismatched donors compared with our case cohort. However, after excluding patients with HLA-mismatched donors from both cohorts, CY/^{T}BU conditioning continued to be associated with a significantly lower incidence of SOS (0% versus 28%; $P = .01$) and lower day +100 mortality (2% versus 12%; $P = .02$) in patients with myelofibrosis, suggesting that our findings were not influenced by this imbalance in donor–recipient HLA matching.

In conclusion, our data indicate that reversing the sequence of conditioning agents from ^{T}BU/CY to CY/^{T}BU before allogeneic HCT is associated with reduced day +100 mortality and a decreased incidence of SOS in patients with myelofibrosis. This reduced hepatotoxicity was likely mediated by reduced

exposure to toxic CY metabolites. This change in conditioning sequence, which requires no additional institutional expertise and uses existing medications and technology, can substantially reduce regimen-related toxicity and early mortality and improve outcomes in patients undergoing allogeneic HCT for myelofibrosis.

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